



Oropharyngeal Gram-negative bacillary carriage in chronic obstructive pulmonary disease: relation to severity of disease

K. J. MOBBS^{*†}, H. K. F. VAN SAENE[†], D. SUNDERLAND[†] AND P. D. O. DAVIES[‡]

Departments of ^{*}Genetics and Microbiology and [†]Medical Microbiology, University of Liverpool, U.K.,

[‡]Cardiothoracic Unit, Broad Green Hospital, Liverpool, U.K.

The prolonged presence of aerobic Gram-negative bacilli (AGNB) in the oropharynx is termed 'carriage'. AGNB carriage rates are low in populations of healthy individuals. Previously, severity of underlying disease has been positively correlated with oropharyngeal AGNB carriage rate. Overgrowth of AGNB at the oropharynx poses a significant risk of endogenous infection in end-stage chronic obstructive pulmonary disease (COPD) patients. The aims of this study were to undertake an epidemiological survey of the oropharyngeal flora of COPD patients and to correlate oropharyngeal carriage of AGNB with severity of disease.

Two oral rinses were obtained, within a 2-day interval, from 40 COPD patients comprising three disease severity groups: 1. mild, 2. moderate and 3. severe. Eighty oral rinses were quantitatively (1:10 dilution series) cultured for AGNB and yeasts using broth enrichment.

The mean AGNB carriage rate was 15%. AGNB carriage rates of 0, 7.7 and 29.4% were observed within the mild, moderate and severe disease groups, respectively. The mean yeast carriage rate was 33.3%. Yeast carriage rates of 33.3, 15.4 and 64.7% were observed within the mild, moderate and severe disease groups, respectively. Carriage of *Staphylococcus aureus* was 5%. Rates of oropharyngeal carriage of AGNB (1/23 vs. 5/17) and yeasts (5/23 vs. 11/17) were significantly higher within the severe disease group than in non-severe disease groups.

Oropharyngeal carriage of AGNB in end-stage COPD patients (forced expiratory volume in 1 sec, FEV₁ < 50% predicted) presents a potential source of Gram-negative endogenous pneumonia. This outcome may be promoted by intubation and some flora-suppressing antibiotic therapies.

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Introduction

Normal oropharyngeal flora consists of a variety of anaerobes (e.g. peptostreptococci, *Veillonella* spp.) at concentrations of approx. 1×10^8 colony forming units (CFU) ml⁻¹ of saliva. Aerobes are represented most prominently by viridans streptococci at approx. 1×10^6 CFU ml⁻¹ of saliva. A variety of potentially pathogenic micro-organisms (PPM) are also found in the 'normal' oropharyngeal flora such as the 'community micro-organisms' *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus* and *Candida* spp. 'Abnormal' oropharyngeal flora are opportunistic aerobic Gram-negative bacilli (AGNB), also termed 'hospital

micro-organisms', e.g. *Klebsiella* spp., *Enterobacter* spp. and *Pseudomonas* spp. All AGNB are considered potential pathogens. Carriage of abnormal oropharyngeal flora appears uncommon in healthy individuals due to the low availability of AGNB-receptor sites within the healthy oral mucosae and the efficacy of innate host factors aimed at clearance of abnormal oropharyngeal flora (1). Underlying illness is thought to be the most important single factor associated with increased availability of AGNB-receptor sites within the oropharyngeal mucosae. The literature suggests that, within a particular subset of patients, prevalence of the AGNB carrier state reflects illness severity (2–4). Literature searches using the mesh words 'chronic obstructive pulmonary disease (COPD)', 'AGNB' and 'oropharynx' revealed three surveys of AGNB carriage within populations with COPD (5–7). AGNB carriage rates varied between 5 and 18%. However, illness severity was not accounted for in these studies. A prospective pilot study was undertaken in a population of 40 outpatients with COPD to evaluate carriage rates of AGNB according to illness severity.

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Correspondence should be addressed to: K. J. Mobbs, Dept. Medical Microbiology, University of Liverpool, P.O. Box 147, Liverpool L69 3GA, U.K. Fax: 00 44 151 706 5805; E-mail: K.J. Mobbs@liv.ac.uk

Materials and Methods

COPD OUTPATIENTS

In total, 40 outpatients were enrolled in this study and fulfilled the following criteria: 1. age older than 16 years; 2. complaints of shortness of breath, wheezing, dyspnoea on effort, cough and sputum production; 3. a clinical diagnosis of COPD made at least once in the past 10 years by a pulmonologist; 4. absence of obvious respiratory infection; and 5. absence of antimicrobial chemotherapy in the 4 weeks preceding sampling.

SEVERITY OF DISEASE

Disease severity was assessed purely on the basis of spirometry. Outpatients were segregated according to the forced expiratory volume in 1 sec (FEV₁) as a percentage of predicted normal values. Subjects classed as suffering mild, moderate and severe disease had FEV₁ values of ≥ 70 , 69–50% and $< 50\%$ of predicted normal values, respectively (8).

MANAGEMENT OF COPD

Outpatients attending clinics and diagnosed as suffering COPD were normally prescribed a β -adrenergic agonist and inhaled steroid. More severe cases may have been prescribed ipratropium bromide. Antibiotic prophylaxis and N-acetylcysteine were considered inappropriate. Antibiotics were prescribed curatively for 1 week only in cases of infectious exacerbations of COPD (9).

SAMPLING

Oropharyngeal samples were obtained via an oral rinse and gargle technique. Patients were presented with 10 ml of sterile isotonic saline and told to rinse and gargle the solution vigorously for 10 sec before expectorating the sample into a sterile receptacle. All samples were processed within 2 h of sampling.

Sampling frequency was twice in 1 week on Tuesdays and Thursdays between 1000 and 1200 hours. Sampling times were maintained to avoid false positive results due to ingestion of contaminated foodstuffs. Clearance of even large numbers of AGNB from the oropharynx is reported to occur within 3 h in healthy individuals.

CULTURE TECHNIQUES (10)

One ml of oropharyngeal sample was diluted 10-fold in Nutrient Broth (Oxoid, Basingstoke, Hampshire, U.K.). Ten-fold dilution series were made in well-trays of 64 (8 \times 8) cups of 1.5 ml volume (WHO trays; Redhill Surgical Co. Ltd, Glasgow, U.K.), containing 0.45 ml Nutrient Broth. To the first cup, a 0.05 ml aliquot of the diluted (1×10^{-1}) sample was added, yielding a further ten-fold dilution

(1×10^{-2}). Subsequent cups were inoculated with 0.05 ml aliquots from the previous cup to a dilution of 1×10^{-9} .

All samples were incubated at 37° C for 18–24 h. Following incubation, the well-trays were scored for turbidity. The number of cups showing turbidity due to bacterial growth indicates the highest log₁₀ concentration of micro-organisms per ml of oropharyngeal sample.

All dilutions displaying a degree of turbidity were subcultured onto MacConkey agar with crystal violet, Columbia blood agar and Sabouraud dextrose agar with lactic acid. All prepared media were obtained from Lab M, Bury, U.K.

AGNB (e.g. *Enterobacteriaceae*, *Pseudomonadaceae* and *Acinetobacter* spp.), yeast, and streptococci and staphylococci were evaluated on MacConkey agar, Sabouraud dextrose agar and Columbia blood agar, respectively.

Morphologically distinct colonies were identified by colonial and cellular morphology. Staphylococci identified through colonial morphology were tested by the slide coagulase test (all coagulase-positive isolates were taken to be the *Staphylococcus aureus*).

All suspected AGNB isolated from MacConkey agar were isolated in pure culture on Columbia blood agar and identified by means of the API 20 E analytical test (bioMérieux sa, Marcy-l'Etoile, France). Identification of microorganisms was based on the same biochemical profile.

DEFINITIONS (11)

Carriage

Carriage, or a carrier state, exists when the same bacterial strain is isolated from at least two consecutive surveillance samples.

Acquisition

The patient is considered to have acquired a micro-organism if only one of two surveillance samples is positive for a micro-organism that differs from the previous and following isolates. Acquisition refers to the transient presence of a micro-organism.

Indigenous Flora

Micro-organisms carried by healthy people at high concentrations ($\geq 1 \times 10^6$ CFU ml⁻¹), e.g. viridans streptococci.

Community PPM

Micro-organisms carried at the oropharynx by varying percentages of the healthy population, e.g. *Staphylococcus aureus* and *Candida albicans*.

TABLE 1. Age and sex distribution among 40 COPD outpatients

Age (years)	COPD outpatients (n)
30-39	3
40-49	3
50-59	11
60-69	12
70-79	10
80-89	1
Men	22
Women	18

Hospital PPM

Includes the aerobic Gram-negative bacilli (AGNB), also termed 'opportunistic' or 'abnormal' micro-organisms.

STATISTICAL ANALYSIS

Statistical analysis was performed using Fisher's exact test. Due to the small numbers involved in some subject groups Yates' correction factor was utilized. Ninety-five percent confidence intervals (95% CI) were calculated using Woolf's approximation.

Results

The group of 40 COPD outpatients was composed of approximately equal numbers of men and women. The modal age group was 60-69 years (Table 1).

A total of 80 oral rinses were obtained (two per patient). All patients carried indigenous flora, represented by viridans streptococci, at concentrations of $\geq 1 \times 10^4$ CFU ml⁻¹. The total mean AGNB carriage rate within the outpatient population was 15.0% (six individuals). AGNB

carriage rates within the mild, moderate and severe disease severity groups were 0, 7.7 and 29.4%, respectively (Table 2). The difference in AGNB carriage rate between non-severe (one in 23, or 4.3%) (including mild and moderate sub-groups) and severe (five in 17, or 29.4%) disease subpopulations was statistically significant (95% CI 0.9567-87.834). Three patients carried oropharyngeal AGNB at concentrations of $\geq 1 \times 10^3$ CFU ml⁻¹, whilst two yielded AGNB at 1×10^4 CFU ml⁻¹ and one patient carried one AGNB at the oropharynx at $\geq 1 \times 10^6$ CFU ml⁻¹ (Table 3). Within 3 days of sampling, this patient with oropharyngeal overgrowth of *Klebsiella oxytoca* developed pneumonia due to the same organism. Total mean yeast carriage rate within the outpatient population was 33.3% (16 individuals). Yeast carriage rates within the mild, moderate and severe disease severity groups were 33.3, 15.4 and 64.7%, respectively (Table 2). Oropharyngeal carriage of yeasts was significantly higher within the severe (11/17) disease group compared with non-severe [5/23] groups (95% CI 1.621-26.881). No patients carried yeasts at concentrations higher than 1×10^4 CFU ml⁻¹ (Table 3).

The total mean rates of acquisition of AGNB and yeasts within the outpatient populations were 45% (18 individuals) and 15% (six individuals), respectively. The modal concentration of acquired AGNB and yeasts was 1×10^1 CFU ml⁻¹. However, six patients acquired oropharyngeal AGNB at concentrations $\geq 1 \times 10^3$ CFU ml⁻¹.

Escherichia coli was the AGNB most frequently recovered from the oropharynx of patients in cases of carriage and acquisition (Table 4). The *Staphylococcus aureus* carriage and acquisition rates were 5 and 17.5%, respectively. *Staphylococcus aureus* was not recovered from any patient at concentrations above 1×10^4 CFU ml⁻¹.

Discussion

The aim of this study within a population of ambulatory COPD outpatients was to execute an epidemiological pilot study to evaluate rates of carriage and acquisition of aerobic Gram-negative bacilli (AGNB) in the oropharynx

TABLE 2. Incidence of carriage of AGNB and yeasts in relation to severity of COPD

Disease severity*	n†	AGNB carriers n (%)	Yeast carriers n (%)
Mild (FEV ₁ \geq 70%)	10	0 (0.0)	3 (33.3)
Moderate (FEV ₁ 69-50%)	13	1 (7.7)	2 (15.4)
Severe (FEV ₁ < 50%)	17	5 (29.4)	11 (64.7)
Total	40	6 (15.0)	16 (40.0)

*Disease severity assessed by spirometry and expressed as a percentage of expected forced expiratory volume in 1 sec (FEV₁).

†Number of outpatients within severity group.

TABLE 3. Number of patients shown to carry and acquire AGNB and yeasts at the given concentrations.

		Log ₁₀ concentrations (CFU ml ⁻¹) of micro-organisms recovered from the oropharynx					
		1	2	3	4	5	≥ 6
AGNB							
Carriage	1	1	1	1	2	—	1
Acquisition	8	4	4	4	1	1	—
Yeasts							
Carriage	3	6	1	6	—	—	—
Acquisition	5	—	1	—	—	—	—

using a sensitive and quantitative multiple survey method. Carriage and acquisition rates for AGNB in the whole study group were 15 and 45%, respectively.

The main finding of this pilot study was that illness severity correlates positively with oropharyngeal carriage rates of both AGNB and yeasts. The group of COPD patients scoring ≥ 50% of predicted FEV₁ (23 individuals) yielded only one carrier of oropharyngeal AGNB (4.3%). The patients scoring <50% of predicted FEV₁ (17 individuals) yielded five carriers of oropharyngeal AGNB (29.4%). Although the population studied here is small, the results prove statistically significant and highlight the need for similar studies performed within larger populations.

The precise physiological mechanisms of abnormal Gram-negative oropharyngeal carriage are as yet unknown. However, healthy individuals are capable of eliminating larger numbers of AGNB from the oropharynx within 3 h of inoculation (12). Such rapid clearance is possible due to the efficacy of seven innate host factors (12–18) and, also, the apparent absence of mucosal receptors for AGNB. It is hypothesized that these receptor molecules are constitutively expressed, but are covered by a protective layer of fibronectin in the healthy mucosae. Significantly increased levels of salivary elastase have been shown to precede Gram-negative colonization of the oropharynx in post-operative

patients (19). It is probable that in individuals suffering both chronic and acute underlying illness, circulating populations of activated macrophages release elastase into mucosal secretions thereby denuding the protective fibronectin layer. It is thought that this hypothetical mechanism is a deleterious consequence of the inflammatory response encountered during and after illness.

It is possible that the yeast carriage rates reported in this paper are also a consequence of the aforementioned mechanisms and, therefore, correlate with disease severity. However, several other factors may be at work within the patient group. Many COPD patients undergo prolonged steroidal chemotherapy, which has been implicated in oral candidiasis (8). Both advanced age and denture wearing have previously been correlated with increased prevalence of yeasts within the oropharynx (20,21). Considering the modal age group of the patients (60–69 years), and the fact that edentate individuals were not discriminated, it is not possible to correlate accurately severity of disease with yeast carriage rate.

Aspiration of contaminated oropharyngeal secretions is the predominant mechanism of nosocomial lower airway infection. *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* are the main causative organisms involved in infectious exacerbations of COPD in patients not undergoing antimicrobial chemotherapy (22–24). Even during the simultaneous carriage of the aforementioned respiratory pathogens and AGNB, *Streptococcus pneumoniae*, *H. influenzae* and *M. catarrhalis* pose a far greater risk of lower airway infection than AGNB due to their higher intrinsic virulence. This is in agreement with the majority of studies on community-acquired pneumonia in COPD patients (25–27).

This pilot study shows a significantly increased AGNB carriage in the severe COPD group scoring <50% predicted FEV₁. Primary endogenous pneumonia due to AGNB within the oropharyngeal flora of the COPD patients on admission varied between 5 and 17% in the community-acquired pneumonia studies within COPD patient populations (5–7).

Many COPD patients will eventually require mechanical ventilation (28). Practically all ventilated COPD patients receive parenteral antimicrobials. Our findings suggest that

TABLE 4. Incidence of acquisition and carriage of different AGNB throughout the COPD outpatient group

Micro-organism	Acquisition* n (%)	Carriage* n (%)
<i>Escherichia coli</i>	8 (20.0)	3 (7.5)
<i>Enterobacter</i> spp.	4 (10.0)	0 (0.0)
<i>Serratia</i> spp.	2 (5.0)	1 (2.5)
<i>Klebsiella</i> spp.	1 (2.5)	1 (2.5)
<i>Proteus</i> spp.	2 (5.0)	1 (2.5)
<i>Citrobacter</i> spp.	0 (0.0)	0 (0.0)
Other	1 (2.5)	0 (0.0)

*Number of outpatients

end-stage COPD patients require systemic antibiotics effective for both 'community' and 'hospital' micro-organisms. The commonly used antimicrobials are all capable of eliminating the three main respiratory pathogens from both the oropharynx and lower airways (29). Most antimicrobials used for this purpose do not attain sufficiently high working concentrations in saliva for bactericidal action against many AGNB (30). Underlying disease, intubation and often suppression of normal indigenous oropharyngeal flora are known to promote overgrowth of AGNB (1). Oropharyngeal overgrowth is an independent risk factor for colonization and infection of the lower airways (31). It is likely that severity of COPD and associated immunosuppression determines progression to superinfection and secondary endogenous AGNB pneumonia (32).

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